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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

June 18,2004

Subject: EPA ID No.: 059102. Chlorpyrifos methyl: Review of a multi-generation reproduction study (2002, MRID No.: 458262001) including comparison of RBC, brain and heart AChE inhibition in adults and nursing pups.

TXR #: 0051811
DP Barcode No.: D289489
Submission No.: S633164
PC Code: 059102

From: John Doherty
ReRegistration Branch III
Health Effects Division 7509C

To: Kathy Monk
Product Manager #52
Special Review and ReRegistration Division 7505C

Through: Catherine Eiden
Branch Chief
ReRegistration Branch III
Health Effects Division 7509C

Conclusions:

ReRegistration Branch III has completed its review of the multi-generation reproduction study (2002, MRID No.: 45826201) and has classified the study as Acceptable/Guideline. The study satisfies the requirement for a series 870.3800 multi-generation reproduction study in rats. A copy of the DER is attached. The study is further identified together with the Executive Summary in the following table.

This study included assessment of the relative inhibition of RBC, brain and heart AChE in the adults parental groups assessed just after the pups were weaned and compared the AChE activity in rat pups taken at day 1, day 4 and day 22 of lactation. The results indicated that the adults demonstrated inhibition of RBC at the low dose of 1 mg/kg/day and heart and brain AChE were only marginally inhibited at 10 mg/kg/day. Only RBC AChE in the rat pups was inhibited and at the high dose of 10 mg/kg/day only. There was no evidence of either heart or brain AChE inhibition in the pups.

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Study	Executive Summary
870.3800. Multi-generation reproduction - rat . Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Laboratory study number 011132, November 12, 2002.	In a 2-generation reproduction study (2002, MRID 45826201) Chlorpyrifos methyl (96.8% a.i., lot # NB05272036) was administered to 30 Crl:CD(SD)IGS BR rats/sex/dose in the diet at nominal dose levels of 0, 1, 3, or 10 mg/kg bw/day. F_0 and F_1 parental animals were dosed for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until necropsy. One litter was produced by each generation. Subsets of 5 litters/group/generation were used to provide offspring for measurement of erythrocyte (RBC), heart, and whole brain acetylcholinesterase (AChE) in 2 pups/sex/litter on postnatal day (PND) 1 and in 1 pup/sex/litter each on PNDs 4 and 22. The parental adults of these litters (5 animals/sex/dose) were used for AChE determinations on PND 22. *Systemic toxicity.* The only indication of parental toxicity was evident as increased absolute adrenal weights in F_0 high-dose males (↑16%) and females (↑14%, both $p<0.05$) and in F_1 high-dose males (↑20%) and mid (↑19%) - and high (30%, all $p < 0.05$) -dose females. These changes correlated with increased incidences and/or severity of cytoplasmic vacuolation of the zona fasciculata.. The lesion was noted in 25/25 high-dose males and females of both generations, in 20/25 mid-dose F_0 females, and in 21/25 and 15/25 F_1 mid-dose males and females, respectively (vs. 11-14/25 male and 4-7/25 female controls). **The LOAEL for parental systemic toxicity is 3 mg/kg bw/day, based on microscopic lesions in the adrenal glands of F_0 females and F_1 animals of both sexes. The NOAEL is 1 mg/kg bw/day.** *Offspring systemic toxicity.* There were no treatment-related effects on the gestation, day 4, or day 21 survival indices, pup body weight, clinical signs during lactation, or gross pathological observations. There were no treatment-related whole litter losses or structural alterations. Decreased absolute and relative (to body) spleen weights (↑15-16%, $p < 0.05$) in F_1 high-dose female weanlings were of dubious toxicological significance. **The LOAEL for systemic offspring toxicity rats is greater than the highest dose tested. The offspring NOAEL is ≥10 mg/kg bw/day.** *Reproductive toxicity.* The male and female mating, conception, and fertility indices, gestation indices, mean precoital intervals, and mean gestation lengths were not affected by test article administration. Offspring sex ratios on LD1, mean postimplantation losses, and mean live litter sizes of the treated groups were similar to those of their respective controls, and there were no whole litter resorptions. No treatment-related effects on mean estrous cycle length during premating and stage of estrous at the time of termination were seen in either generation. There were no treatment-related effects on sperm morphology from treated males, and there was no evidence of treatment-related depletion of "small" and/or "growing" ovarian follicles in the high-dose F_1 females as evaluated quantitatively. Sexual maturation of F_1 weanlings was not affected by treatment, and there were no treatment-related gross findings related to the reproductive organs of the parental animals or offspring. **The reproductive LOAEL is greater than the highest dose tested. The NOAEL is ≥10 mg/kg bw/day.** *RBC AChE inhibition.* In adult rats, RBC AChE was significantly inhibited in low (~20%, F_0 males only) and in mid- (~61-69%) and high-dose (~81-94%) parental animals of both sexes and generations. In the offspring, RBC AChE was inhibited in high-dose F_1 males and females on PND 1 (~36 and 23% inhibition, respectively) and on PND 4 (~30 and 22% inhibition, respectively), in high-dose F_2 males on PND 1 (~26% inhibition), and in high-dose F_2 females on PND 4 (~25% inhibition). RBC AChE was increased by approximately 20-29% in high-dose F_1 males and females on PND 22. **The LOAEL for RBC AChE inhibition was 1 mg/kg bw/day (the lowest dose tested), based on >20% inhibition in adult F_0 males. The NOAEL was not identified.** The LOAEL for offspring RBC AChE was 10 mg/kg/day and the NOAEL was 3 mg/kg/day meaning that the adults were more sensitive. *Brain AChE inhibition.* Brain AChE in adults was 10-19% decreased in high-dose F_0 and F_1 groups for both sexes. No treatment-related effects were seen on brain AChE in offspring. **The LOAEL for brain AChE inhibition is 10 mg/kg/day. The NOAEL is 3 mg/kg/day.** *Heart AChE inhibition.* AChE activity was significantly inhibited in high-dose parental F_0 males (~25%) and females (22%) and in high-dose F_1 females (~22% inhibition). There were no treatment-related effects in offspring. **The LOAEL for heart AChE inhibition was 10 mg/kg bw/day, based on adult F_0 males and females and adult F_1 females.** This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 2-generation reproductive study [OPPTS 870.3800; OECD 416] in rats. Although, there were several deficiencies in the conduct of this study, it is the opinion of the reviewer that the study satisfied the *intent* of the guideline. One study deficiency is that plasma ChE was not assessed.

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DATA EVALUATION RECORD

CHLORPYRIFOS METHYL/059102

STUDY TYPE: REPRODUCTION AND FERTILITY EFFECTS STUDY - RAT [OPPTS 870.3800 (§83-4); OECD 416]

MRID 45826201

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 03-24

Robert H. Ross

Primary Reviewer:
Donna L. Fefee, D.V.M.
Signature: for D. L. Fefee
Date: OCT 0 8 2003

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
Signature: Carol S Wood
Date: OCT 0 8 2003

Robert H. Ross, M.S., Group Leader
Signature: Robert H. Ross
Date: OCT 0 8 2003

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L. A. Wilson
Date: OCT 0 8 2003

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: J. Doherty, Ph.D.
Reregistration Branch 3, Health Effects Division (7509C)
EPA Work Assignment Manager: G. Dannan, Ph.D.
Registration Branch 3, Health Effects Division (7509C)

Signature: [signature]
Date June 8, 2004
Signature: Glenn A. Dannan
Date 6/9/04

Template version 11/01

DATA EVALUATION RECORD
TXR#: 0051811

STUDY TYPE: Reproduction and Fertility Effects Study - Rat; OPPTS 870.3800 (§83-4); OECD 416.

PC CODE: 059102

DP BARCODE: D289489
SUBMISSION NO.: S633174

TEST MATERIAL (PURITY): Chlorpyrifos-methyl (96.8% a.i.)

SYNONYMS: O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate; RELDAN™ insecticide; RELDAN™ insecticide F.

CITATION: Carney, E., K. Stebbins, B. Marable, et al. (2002) Chlorpyrifos-methyl: two-generation dietary reproduction toxicity study in CD rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Laboratory study number 011132, November 12, 2002. MRID 45826201. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268.

EXECUTIVE SUMMARY: In a 2-generation reproduction study (2002, MRID 45826201) Chlorpyrifos methyl (96.8% a.i., lot # NB05272036) was administered to 30 Crl:CD(SD)IGS BR rats/sex/dose in the diet at nominal dose levels of 0, 1, 3, or 10 mg/kg bw/day. F_0 and F_1 parental animals were dosed for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until necropsy. One litter was produced by each generation. Subsets of 5 litters/group/generation were used to provide offspring for measurement of erythrocyte (RBC), heart, and whole brain acetylcholinesterase (AChE) in 2 pups/sex/litter on postnatal day (PND) 1 and in 1 pup/sex/litter each on PNDs 4 and 22. The parental adults of these litters (5 animals/sex/dose) were used for AChE determinations on PND 22.

Systemic toxicity. The only indication of parental toxicity was evident as increased absolute adrenal weights in F_0 high-dose males (↑16%) and females (↑14%, both $p<0.05$) and in F_1 high-dose males (↑20%) and mid (↑19%) - and high (30%, all $p < 0.05$) -dose females. These changes correlated with increased incidences and/or severity of cytoplasmic vacuolation of the zona fasciculata.. The lesion was noted in 25/25 high-dose males and females of both generations, in 20/25 mid-dose F_0 females, and in 21/25 and 15/25 F_1 mid-dose males and females, respectively (vs. 11-14/25 male and 4-7/25 female controls). **The LOAEL for parental systemic toxicity is 3 mg/kg bw/day, based on microscopic lesions in the adrenal glands of F_0 females and F_1 animals of both sexes. The NOAEL is 1 mg/kg bw/day.**

Offspring systemic toxicity. There were no treatment-related effects on the gestation, day 4, or day 21 survival indices, pup body weight, clinical signs during lactation, or gross pathological observations. There were no treatment-related whole litter losses or structural alterations. Decreased absolute and relative (to body) spleen weights (↑15-16%, $p < 0.05$) in F_1 high-dose female weanlings were of dubious toxicological significance. **The LOAEL for systemic offspring toxicity rats is greater than the highest dose tested. The offspring NOAEL is ≥10 mg/kg bw/day.**

Reproductive toxicity. The male and female mating, conception, and fertility indices, gestation indices, mean precoital intervals, and mean gestation lengths were not affected by test article administration. Offspring sex ratios on LD1, mean postimplantation losses, and mean live litter sizes of the treated groups were similar to those of their respective controls, and there were no whole litter resorptions. No treatment-related effects on mean estrous cycle length during premating and stage of estrous at the time of termination were seen in either generation. There were no treatment-related effects on sperm morphology from treated males, and there was no evidence of treatment-related depletion of "small" and/or "growing" ovarian follicles in the high-dose F_1 females as evaluated quantitatively. Sexual maturation of F_1 weanlings was not affected by treatment, and there were no treatment-related gross findings related to the reproductive organs of the parental animals or offspring. **The reproductive LOAEL is greater than the highest dose tested. The NOAEL is ≥10 mg/kg bw/day.**

RBC AChE inhibition. In adult rats, RBC AChE was significantly inhibited in low (~20%, F_0 males only) and in mid- (~61-69%) and high-dose (~81-94%) parental animals of both sexes and generations. In the offspring, RBC AChE was inhibited in high-dose F_1 males and females on PND 1 (~36 and 23% inhibition, respectively) and on PND 4 (~30 and 22% inhibition, respectively), in high-dose F_2 males on PND 1 (~26% inhibition), and in high-dose F_2 females on PND 4 (~25% inhibition). RBC AChE was increased by approximately 20-29% in high-dose F_1 males and females on PND 22. **The LOAEL for RBC AChE inhibition was 1 mg/kg bw/day (the lowest dose tested), based on >20% inhibition in adult F_0 males. The NOAEL was not identified.** The LOAEL for offspring RBC AChE was 10 mg/kg/day and the NOAEL was 3 mg/kg/day meaning that the adults were more sensitive.

Brain AChE inhibition. Brain AChE in adults was 10-19% decreased in high-dose F_0 and F_1 groups for both sexes. No treatment-related effects were seen on brain AChE in offspring. **The LOAEL for brain AChE inhibition is 10 mg/kg/day. The NOAEL is 3 mg/kg/day.**

Heart AChE inhibition. AChE activity was significantly inhibited in high-dose parental F_0 males (~25%) and females (22%) and in high-dose F_1 females (~22% inhibition). There were no treatment-related effects in offspring. **The LOAEL for heart AChE inhibition was 10 mg/kg bw/day, based on adult F_0 males and females and adult F_1 females.**

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 2-generation reproductive study [OPPTS 870.3800; OECD 416] in rats. Although, there were several deficiencies in the conduct of this study, it is the opinion of the reviewer that the study satisfied the *intent* of the guideline. One study deficiency is that plasma ChE was not assessed.

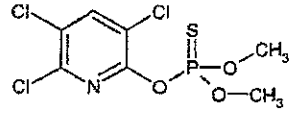
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Chlorpyrifos-methyl

Description: Amber liquid
Lot #: NB05272036
Purity: 96.8% a.i.
Compound Stability: Not available
CAS # of TGAI: 005598-13-0
Structure:



2. Vehicle and/or positive control: The test article was incorporated into the diet (see below). There was no positive control.

3. Test animals:

Species:	Rat	
Strain:	Crl:CD(SD)IGS BR	
Age at study initiation:	(P) 6 wks; (F_1) 3-5 wks	
Wt. at study initiation:	P (measured on day -2): Males: 163.3-202.6 g; Females: 132.5-163.6 g F_1: Males: 47.6-167.9 g; Females: 48.0-134.4 g	
Source:	Charles River Laboratories Inc. (Portage, Michigan)	
Housing:	Individually in suspended stainless steel cages with wire-mesh floors except during mating when males and females were cohoused (1:1) and from GD 19 through lactation when females and litters were housed in plastic cages with nesting material	
Diet:	LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form *ad libitum*	
Water:	Municipal water *ad libitum*	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Approximately 2 weeks	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: August 10, 2001; end: April 17, 2002.

2. **Mating procedure:** Each female was caged with a male from the same treatment group for up to 14 consecutive days. Mating was confirmed by observation of a vaginal copulatory plug and/or the presence of spermatozoa in a daily vaginal smear; this day was designated gestation day (GD) 0, and mated females were returned to individual housing. If there was no evidence of mating after 14 days, the animals were returned to individual housing with no further opportunity for mating. F_1 and F_2 generations were produced with one litter per generation. Sibling matings were avoided.

3. **Study schedule:** The F_0 and F_1 parental animals were given test diets for approximately 10 weeks before they were mated, during mating, gestation, and lactation, and until sacrifice. The day of birth was designated postnatal day (PND) and lactation day (LD) 0, and selection of F_1 parental animals was made on PND 21, at which time the selected animals were weaned onto the same diets as their parents.

4. **Animal assignment:** Animal assignment is given in Table 1. F_0 parental animals were assigned to treatment groups according to body weight using a computerized stratified randomization procedure designed to give uniform group means and standard deviations. For the F_1 generation, an unspecified random method was used to select at least one male and one female pup from each litter.

   Subsets of 5 F_0 and F_1 parental animals/sex/dose and their progeny were designated for measurement of acetylcholinesterase activity by selecting the first 5 litters per group that were born during the work week and contained adequate numbers of male and female pups (minimums of 5 per sex for the F_1 litters and 4 per sex for the F_2 litters). A computerized random selection procedure was used to choose 2 pups/sex/litter for the determinations done on PND 1 and to choose 1 pup/sex/litter for the determinations done on PND 4 and PND 22. When possible, pups for the PND 4 determinations were chosen from among any surplus pups that were to be culled (see litter observations).

TABLE 1. Animal assignment				
Test group	**Control**	**Low-dose**	**Mid-dose**	**High-dose**
Nominal Dose [a] (mg/kg/day)	**0**	**1**	**3**	**10**
Parental Animals				
Number of males/females:				
F_0 generation	30/30	30/30	30/30	30/30
F_1 generation	30/30	30/30	30/30	30/30
Acetylcholinesterase determinations				
Adults: F_0 generation	5/sex	5/sex	5/sex	5/sex
F_1 generation	5/sex	5/sex	5/sex	5/sex
PND 1: F_1 generation	10/sex	10/sex	10/sex	10/sex
F_2 generation	10/sex	10/sex	10/sex	10/sex
PND 4: F_1 generation	5/sex	5/sex	5/sex	5/sex
F_2 generation	5/sex	5/sex	5/sex	5/sex
PND 22: F_1 generation	5/sex	5/sex	5/sex	5/sex
F_2 generation	5/sex	5/sex	5/sex	5/sex

Data taken from pp. 25 and 34, MRID 45826201.

[a] Dietary concentrations were adjusted at each mix according to the most recent body weight and food consumption data from the current study or historical controls.

5. **Dose selection rationale:** The dose levels were selected based on the results from previous studies with the test material or with Chlorpyrifos, a compound that is structurally related to Chlorpyrifos methyl.

In a 3-generation reproductive toxicity study conducted in 1975, *Chlorpyrifos-methyl* was administered to Sprague-Dawley rats in the diet at dose levels of 0, 1, or 3 mg/kg bw/day, and each generation produced two litters. In parental animals of the third generation, significant inhibition of plasma cholinesterase activity occurred in both sexes at both dose levels, and significant inhibition of erythrocyte cholinesterase activity was seen in high-dose males and all females; however, there was no effect on brain cholinesterase activity. There were no treatment-related effects on pup survival or body weight. Fertility indices of the high-dose animals were slightly decreased, but the differences were not statistically significant, and later re-analysis of the female reproductive parameters found that all values fell within the typical range of normal variation.

In a 2-generation reproductive toxicity study with *Chlorpyrifos (an ethyl analog of chlorpyrifos methyl)*, dietary administration of the compound resulted in the following parental effects: significantly decreased plasma and erythrocyte cholinesterase activities in F_0 and F_1 males and females at 1.0 and 5.0 mg/kg bw/day; significantly decreased brain cholinesterase activity in F_0 and F_1 males and females at 5.0 mg/kg bw/day (42-52% of controls); and microscopic alterations in the zona fasciculata of the adrenal gland at 5.0

mg/kg bw/day in females from at least one generation. At 5.0 mg/kg bw/day, pup body weights and survival were significantly decreased in F_1 litters but not in F_2 litters. There were no treatment-related effects on fertility.

In developmental neurotoxicity studies with *Chlorpyrifos*, administration of the compound by gavage to pregnant Sprague-Dawley rats at dose levels of 0, 0.3, 1.0, or 5.0 mg/kg bw/day from GD 6 through PND 10 resulted in cholinesterase inhibition in high-dose offspring and in all treated dams. Cholinesterase inhibition was greatest in erythrocytes and plasma followed by brain and then heart. Cholinesterase activities tended to be decreased in late gestation and the first few postnatal days, with values similar to those of controls occurring by approximately PND 5.

Dose levels of 1, 3, and 10 mg/kg bw/day were selected for the current study with the expectation that 10 mg/kg bw/day would result in parental toxicity, and that the lower dose levels would provide dose-response data and establish a NOAEL.

6. **Dosage preparation and analysis:** Test diets were prepared by serial dilution of a 400 ppm (week 1) or 800 ppm premix with additional feed to attain the appropriate concentrations. Premixes were prepared at unspecified intervals that were based on stability data. Diets were prepared weekly during premating, and at least once during mating, gestation, lactation, and the interval between weaning and termination. In order to achieve the nominal daily doses of test material, separate diet formulations were prepared for each sex, and the concentrations of the test substance in the diet were adjusted at each mix according to the most recent body weight data and food consumption data from either the prior week of the current study or from historical controls (during lactation). *The study report did not describe the storage conditions for the diets or premixes.* According to the study report, stability analysis from a previously conducted subchronic toxicity study indicated that the test material was stable in rodent diet for up to 14 days under *unspecified* storage conditions. In the current study, stability was evaluated using 2.5, 50, and 5000 ppm mixtures of the test material in feed that were stored at an unspecified temperature for 9, 29, and 44 days. On four separate occasions during the study, samples of the premix and the treated diets for all groups were analyzed for concentration. Diets for low-dose females and high-dose males (2 occasions) or high-dose females (2 occasions) were evaluated for homogeneity using samples taken from the top and bottom of the container at 3 different positions.

Results:

Homogeneity analysis: All of the measured values for the individual sub-samples of the high-dose diets were within ±5% of their respective means, and the relative standard deviations of all 4 high-dose diet mixes were ≤3.0%. Most of the measured values for the individual sub-samples from 4 diet mixes for low-dose females were within ±8% of their respective means, but on two occasions the measured value for one of the sub-samples differed from the mean by ≥15%. The relative standard deviations for the 4 low-dose female diet mixes were 7.47, 3.46, 2.59, and 8.45%.

Stability analysis: After 9, 29, and 44 days of storage (*exact condition unspecified)*, the measured concentrations of the 2.5 ppm mixture were 85.9, 84.1, and 82.2% of initial, respectively, the measured concentrations of the 50 ppm diet were 107, 93.7, and 120% of initial,

respectively, and the measured concentrations of the 5000 ppm diet were 116, 99.3, and 110% of initial, respectively. *Thus, although the exact conditions of storage were not specified in this report, the stability data indicate that the test material was stable in the diet preparations.*

Concentration analysis: Actual concentrations of the 4 analyzed samples of pre-mix were all within ±10% of target. Actual concentrations of the analyzed samples of the diet mixes were all within ±16% of target, with most falling within ±10% of target.

In summary. The analytical data indicated that the mixing procedure was adequate and that the variance between target and actual dietary concentrations was acceptable. The variance between nominal and actual dosage to the study animals was not reported.

C. OBSERVATIONS:

1. **Parental animals:** Animals were observed cage-side twice daily for morbidity, mortality, or clinical signs of toxicity or ill health. Males were given clinical examinations once prior to treatment and weekly throughout the study. Females were given clinical examinations once prior to treatment, weekly during premating and mating, on GD 0, 7, 14, and 21, and on LD 0, 1, 4, 7, 14, and 21 (or weekly in the case of females that failed to mate or did not deliver litters). Body weight and food consumption for males were recorded weekly throughout the study, except food consumption was not measured during mating. Body weight and food consumption for females were recorded weekly during premating. Mated females were weighed on GD 0, 7, 14, and 20, and females that littered were weighed on LD 1, 4, 7, 14, and 21. Food consumption for these females was measured over the same intervals with the addition of LD 11, 17, and 19. Daily vaginal smears were prepared from all adult F_0 and F_1 females beginning 3 weeks prior to mating and continuing until evidence of mating was observed or until the end of the 2-week mating interval.

2. **Litter observations:** Litter observations were made as shown (X) in Table 2. Anogenital distance was not measured. On LD 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible) using a computerized random selection procedure, and excess pups (other than those designated for PND 4 acetylcholinesterase determinations) were killed via intraperitoneal injection with Socumb euthanasia solution and were discarded. Dead pups and those sacrificed moribund were sexed and examined grossly for external and visceral abnormalities. Pups were weaned on LD 21.

   In addition, sexual maturation was evaluated in the F_1 weanlings selected as parental animals. This was done by examining the animals daily for either vaginal opening (beginning on LD 28) or preputial separation (beginning on LD 35), as appropriate, and recording the age and body weight of each animal at the time of landmark acquisition.

TABLE 2. F_1 / F_2 Litter observations							
Observation	Time of observation (lactation day)						
	Day 0	Day 1	Day 4 [a]	Day 4 [b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X		X	X	X
Number of dead pups	X	X	X		X	X	X
Sex of each pup (M/F)		X	X	X	X	X	X
Individual pup weights		X	X	X	X	X	X
External alterations	X	X	X		X	X	X
Clinical signs	X	X	X		X	X	X

Data taken from text, p. 29, MRID 452826201.

[a] Before standardization (culling).

[b] After standardization (culling).

3. Postmortem observations:

a. Parental animals: As soon as possible after the last litter was weaned, all surviving adults (except those selected for acetylcholinesterase determinations) were sacrificed via decapitation under carbon dioxide anesthesia and subjected to a complete necropsy. At termination, vaginal smears were prepared from all F_0 and F_1 females. Gross necropsy included examination of external surfaces, orifices, cervical, thoracic, and abdominal viscera, brain, pituitary, and eyes (in situ). For females, the number of implantation sites was determined following sodium sulfide staining of the uterus, which was then rinsed and retained in 10% neutral buffered formalin.

Immediately after euthanasia, sperm was collected from the right cauda epididymis of all F_0 and F_1 males, incubated, and evaluated for total and progressive motility using computer assisted motion analysis (IVOS). Air-dried smears were prepared, and 200 sperm per sample were evaluated and classified as either normal or abnormal according to criteria by Filler (1993). The left cauda epididymis and left testis of all males were weighed and then frozen for later determination of homogenization-resistant spermatids and caudal epididymal sperm reserve (initially performed for control and high-dose males only).

The indicated (X) organs or tissues were collected and preserved in 10% neutral buffered formalin or in Bouin's fixative (for testes and ovaries), and selected organs (XX), in addition, were weighed. Histopathological examination was conducted on selected tissues (*) from all necropsied adults of the control and high-dose groups, adrenal glands and gross lesions from all low- and mid-dose animals, and reproductive organs from any animal that exhibited signs of reduced fertility.

Ovaries from 15 randomly selected F_1 females per group were examined to obtain primordial (small and growing) follicle counts; section selection and the number of sections examined were not reported.

Terminal body weights and organ weights were not recorded from animals that died or

were sacrificed moribund, and, for males, both right and left testes and epididymides were preserved in Bouin's fixative without collection of sperm.

	MALE REPRODUCTIVE		DIGESTIVE		URINARY
XX*	Testes [a]	X	Oral tissues	XX*	Kidneys
XX*	Epididymides [a]	X	Tongue	X	Urinary bladder
XX*	Prostate	X	Salivary glands		
XX*	Seminal vesicles [b]	X	Esophagus		RESPIRATORY
X*	Coagulating glands	X	Stomach	X	Trachea
		X	Duodenum	X	Lungs
	FEMALE REPRODUCTIVE	X	Jejunum	X	Nasal tissues
XX*	Ovaries	X	Ileum	X	Larynx
X*	Oviducts	X	Cecum		
XX*	Uterus [c]	X	Colon		NEUROLOGIC
X*	Cervix	X	Rectum	XX	Brain (multiple sections)
X*	Vagina	XX*	Liver	XX*	Pituitary
		X	Pancreas	X	Spinal cord (3 levels)
				X	Peripheral nerve
	GLANDULAR		CARDIOVASC./HEMAT.	X	Eyes (with optic nerve)
XX*	Adrenal gland	X	Aorta		
X	Lacrimal gland	X	Heart		OTHER
X	Parathyroids	X	Bone marrow	X	Bone (with joint)
XX	Thyroids [d]	X	Lymph nodes	X	Skeletal muscle
X	Auditory sebaceous glands	XX	Spleen	X	Skin and subcutis
X*	Mammary gland (females only)	X	Thymus	X*	Gross lesions
				X	Physical identifier (transponder)

Data taken from text, pp. 30-33, Table 1, p. 72, MRID 45826201.

[a] Only the right testis and epididymis were examined histologically.

[b] The seminal vesicles were weighed with the coagulating glands and seminal fluid.

[c] The uterus was weighed with the oviducts and cervix.

[d] The thyroid glands were weighed following fixation.

b. Offspring: Pups found dead during lactation or sacrificed moribund were sexed, examined for gross external or visceral defects, and retained in 10% neutral buffered formalin. On PND 22, three randomly selected weanlings/sex/litter were sacrificed via decapitation under carbon dioxide anesthesia and subjected to a complete necropsy that included examination of external surfaces, orifices, cervical, thoracic, and abdominal viscera, brain, pituitary, and eyes (in situ). At necropsy, the brain, spleen, uterus, and thymus from one weanling/sex/litter were weighed, and representative sections of the weighed organs along with the adrenals and any gross lesions from the same one weanling/sex/litter were retained in 10% neutral buffered formalin for possible future histopathological examination.

The remaining F_1 and F_2 offspring not selected as parental animals, for necropsy, or for use in PND 22 acetylcholinesterase determinations were sacrificed via carbon dioxide asphyxiation and discarded.

c. Acetylcholinesterase Activity: Erythrocyte, heart, and brain acetylcholinesterase activities were determined for both generations using 5 parental adults/sex/dose on

approximately PND 22, 2 pups/sex/litter (10 pups/sex/dose) on PND 1, and 1 pup/sex/litter (5 pups/sex/dose) on both PND 4 and PND 22. The samples taken on PND 1 were pooled by sex and litter.

Blood was collected from fasted adults and unfasted 22-day old weanlings by orbital sinus blood sampling under carbon dioxide anesthesia, and the animals were then returned to a deep plane of anesthesia for euthanasia via decapitation. Unfasted one- and 4-day old pups were anesthetized with intraperitoneal pentobarbital and heparin for midline thoracotomy, blood collection from the aortic arch, and subsequent euthanasia via exsanguination.

Blood samples were collected into heparinized tubes and centrifuged at 2000 RPM for 10 minutes at room temperature. The packed cells were then resuspended in saline, centrifuged, and diluted with homogenization buffer [1.0% (v/v) Triton X-100 in 0.1 M sodium phosphate buffer; pH 8.0] at a final dilution of 1:20. Immediately following euthanasia, the heart and the left sagittal half of the brain for adults or whole brain for pups were removed and cleaned. Tissue samples were then rinsed in ice-cold phosphate buffered saline, blotted, weighed, minced, and homogenized in ice-cold homogenization buffer as 1:5 dilutions for heart tissue and 1:10 solutions for brain tissue. During homogenization, 10-20 second bursts of homogenization were alternated with cooling on ice, and the tissues were re-minced. The tissue homogenates were centrifuged at 8000 x g for 10 minutes at 4° C, and the supernatants were collected.

Six-µL aliquots of supernatant or erythrocyte preparations were pre-incubated at 37° C. for 5 minutes in 300 µL of Ellman's buffer containing 0.1 mM of a butyrylcholinesterase inhibitor (iso-OMPA). A 50 µL volume of 8.5 mM of acetylthiocholine in 0.1 M sodium phosphate buffer was added (pH 8.0; final substrate concentration 1.19 mM), followed by a one-minute mixing process. A Hitachi 914 Clinical Chemistry Autoanalyzer was used to monitor and record the absorbance at 415 nm every 12 seconds for 2 minutes, and the calculated activity of each sample was adjusted for tissue dilution. A commercially available kit (Pierce BCA method; BioRad Laboratories) was used to determine the protein content of frozen supernatants.

D. DATA ANALYSIS:

1. **Statistical analyses:** Parental and offspring body weights, maternal body weight gains, food consumption, sperm counts, percentages of total and progressively motile sperm, estrous cycle length, ovarian follicle counts, and absolute and relative organ weights were first analyzed using Bartlett's test for equality of variance. Homogenous data were then analyzed using a parametric analysis of variance (ANOVA), followed by Dunnett's test if the results of the ANOVA were significant. Non-homogenous data were analyzed using a nonparametric ANOVA, followed by the Wilcoxon Rank-Sum test with Bonferroni's correction if the results of the ANOVA were significant. Gestation length, age at vaginal opening or preputial separation, precoital intervals, and litter size were analyzed using a nonparametric ANOVA, followed by the Wilcoxon Rank-Sum test with Bonferroni's correction if the results of the ANOVA were significant. A sequential outliers test by Grubbs was used to identify statistical outliers, which were excluded if there was a sound scientific reason. The mating,

conception, fertility, and gestation indices were analyzed using the Fisher exact test with Bonferroni's correction. Sperm morphology data were arcsine-transformed prior to analysis using a parametric ANOVA. Offspring sex ratios were analyzed using the binomial distribution test. Offspring survival indices, postimplantation loss, and other offspring incidence data were analyzed using a censored Wilcoxon test as modified by Haseman and Hoel with Bonferroni's correction. Acetylcholinesterase activities were expressed as percentages of controls and analyzed separately by sex using Dunnett's test.

All tests used a significance level of $p<0.05$ except for Bartlett's test for equality of variance and the sequential outliers test which used significance levels of $p<0.01$ and $p<0.02$, respectively.

The reviewer considers the analyses used to be appropriate.

2. **Indices:**

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Female mating index (%) = (No. females with evidence of mating)/(No. paired)×100.

Male mating index (%) = (No. males with evidence of mating)/(No. paired)×100.

Female conception index (%) = (No. females with evidence of delivering a litter)/(No. mated)×100.

Male conception index (%) = (No. males siring a litter)/(No. mated)× 100.

Female fertility index (%) = (No. females with evidence of delivering a litter)/(No. paired)×100.

Male fertility index (%) = (No. males siring a litter)/(No. paired)×100.

Gestation index (%) = (No. females delivering a viable litter)/(No. females delivering a litter)×100.

Postimplantation loss (%) = (No. implants - No. viable offspring)(/No. implants)×100.

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

Gestation survival index (%) = (No. pups born alive)/(Total No. pups born)×100.

Day 1 or 4 pup survival index (%) = (No. viable pups on day 1 or 4 [pre-cull])/(No. born alive)×100.

Day 7, 14, or 21 pup survival index (%) = (No. viable pups on day 7, 14, or 21)/(No. live on day 4 [post-cull])×100.

3. **Historical control data:** Historical control data were not provided.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical signs:** There were no spontaneous deaths or unscheduled sacrifices of F_0 adults or F_1 females. One F_1 control male died on test day 50 with no prior abnormal clinical signs, and one F_1 low-dose male was sacrificed moribund on test day 106 with clinical observations of red urine, urine soiling, an ungroomed appearance, and blood in the cage.

   There were no treatment-related clinical signs. Among survivors, red urine was noted from an additional 1-3 males from the F_0 high-dose and F_1 control, mid-, and high-dose groups. One F_1 *control* female had muscle tremors and tonic convulsions, and 1 F_1 low-dose male exhibited repetitive behavior (continuously tasting the air). Other reported clinical signs included malocclusion incisors, injuries, dermatitis, flaking/scaling, hair loss, staining around the eyes/nose/mouth or perineum, and palpable swelling or masses. All of these were seen at low incidences with no evidence of a dose-response.

2. **Body weight and food consumption:**

a. **Premating:** Selected body weight and food consumption data for F_0 and F_1 generation adults during premating are given in Tables 3 and 4, respectively. The absolute body weight, body weight gain, and food consumption of the treated groups were similar to those of their respective controls. Statistically significant 6-7% increases in the food consumption of the F_1 mid- and high-dose females during days 1-8 occurred without a dose-response and were of insufficient magnitude to be considered biologically significant.

b. **Gestation and lactation periods:** There were no treatment-related effects on the absolute body weight or body weight gain of the F_0 and F_1 females during gestation and lactation. Mean food consumption of the treated F_0 and F_1 females during gestation and lactation was generally similar to that of their respective controls. Statistically significant 13% increases in the LD 19-21 food consumption values for mid- and high-dose F_0 dams were not considered treatment-related due to the lack of a dose-response. The increased mean food consumption of high-dose F_1 females during LD 19-21 (111% of controls; $p<0.05$) may have been treatment-related but was of insufficient magnitude and duration to be considered biologically significant and adverse.

TABLE 3. Mean body weight and food consumption data of F_0 adults during pre-mating [a]

Observations/Study day or interval		Target dose level (mg/kg bw/day)			
		Control	1	3	10
F_0 Generation Males					
Mean body weight (g)	Day -2	183.4±9.4	183.4±9.3	183.4±9.6	183.4±9.5
	Day 20	328.4±29.3	331.3±20.8 [b]	334.7±22.5	330.9±24.8
	Day 41	415.1±49.4	422.4±32.3	425.8±35.2	422.0±37.0
	Day 69 (end of premating)	480.7±61.3	488.7±44.3	495.0±43.2	488.0±46.3
	Day 118	547.3±73.3	548.4±55.6	556.5±51.9	549.6±51.6
Premating weight gain (g) [c]	Days -2 to 69	297.3	305.3	311.6	304.6
Mean daily food consumption (g/animal/day) [d]					
	Days 1-8	24.5±1.9	24.2±1.7	24.6±1.7	24.7±1.8
	Days 29-36	26.5±3.6	26.7±2.2	26.9±2.4	26.8±2.5
	Days 64-71	25.5±3.0	25.1±2.4	25.6±2.0	25.7±2.7
F_0 Generation Females					
Mean body weight (g)	Day -2	147.8±7.4	147.7±7.4	147.8±7.1	147.9±7.1
	Day 20	208.5±17.6	208.4±15.1	212.7±15.9	209.5±12.8
	Day 41	247.0±24.3	247.0±18.2	253.8±24.9	246.2±14.9
	Day 69	268.4±24.7	268.7±22.6	276.2±23.4	267.8±17.0
Premating weight gain (g) [c]	Days -2 to 69	120.6	121.0	128.4	119.9
Mean daily food consumption (g/animal/day) [d]					
	Days 1-8	17.5±1.6	17.3±1.3	17.5±1.7	17.4±1.3
	Days 29-36	18.7±1.9	18.8±2.0	18.3±2.3	18.1±1.6
	Days 64-71	17.5±1.5	17.4±1.7	17.3±1.4	17.0±1.4

Data taken from Tables 13, 14, 21, and 22, pp. 97-98, 99, 107-108, and 109, respectively, MRID 45826201.

[a] Values given as Mean ± Standard Deviation. Unless otherwise noted, group size was n = 30 for all groups.

[b] n = 29, due to measuring error.

[c] Calculated by reviewer from group mean body weight data and not subjected to statistical analysis.

[d] Statistical outliers were excluded; n = 25-30.

TABLE 4. Mean body weight and food consumption data of F_1 adults during pre-mating [a]

Observations/Study day		Nominal dose level (mg/kg bw/day)			
		Control	1	3	10
F_1 Generation Males					
Mean body weight (g)	Day 1	126.1±16.2	124.6±20.4	128.6±22.2	128.5±14.6
	Day 20	291.4±31.2	283.9±34.6	297.1±31.4	298.0±23.6
	Day 41	428.7±46.1	418.1±37.4	437.0±40.7	429.1±31.9
	Day 69 (end of premating)	512.1±61.4 [b]	507.0±44.1	528.1±55.8	515.7±41.0
	Day 125	613.8±79.9 [b]	603.0±52.1 [b]	635.5±73.1	611.0±51.6
Premating weight gain (g) [c]	Days 1-69	386.0	382.4	399.5	387.2
Mean daily food consumption (g/animal/day) [d]					
	Days 1-8	20.7±2.4	20.6±1.9	21.3±2.0	21.4±1.8
	Days 29-36	28.8±2.5	28.5±2.2	29.8±2.8	29.2±2.1
	Days 64-71	28.3±3.0	28.4±2.5	29.3±3.1	28.2±2.1
F_1 Generation Females					
Mean body weight (g)	Day 1	104.5±14.0	110.0±13.7	108.8±16.0	109.8±10.7
	Day 20	189.8±19.4	193.9±18.0	192.8±18.2	196.8±15.6
	Day 41	248.3±30.0	251.7±23.7	248.7±21.7	255.6±23.1
	Day 69	281.5±37.8	286.3±27.5	280.0±20.0	291.7±29.5
Premating weight gain (g) [c]	Days 1-69	177.0	176.3	171.2	181.9
Mean daily food consumption (g/animal/day) [d]					
	Days 1-8	16.3±1.8	17.2±1.7	17.4±1.4 * (107) [e]	17.2±0.9 * (106)
	Days 29-36	19.5±2.2	20.1±2.3	19.9±2.0	20.0±2.1
	Days 64-71	19.1±2.3	19.0±2.0	18.6±1.5	19.6±2.0

Data taken from Tables 17, 18, 27, and 28, pp. 102-103, 104, 114-115, and 116, respectively, MRID 45826201.

[a] Values given as Mean ± Standard Deviation. Unless otherwise noted, group size was n = 30 for all groups.

[b] n = 29, due to death or moribund sacrifice.

[c] Calculated by reviewer from group mean body weight data and not subjected to statistical analysis.

[d] Statistical outliers were excluded; n = 26-30.

[e] Numbers in parentheses equal percent of control, calculated by reviewer.

Significantly different from controls: * $p<0.05$.

3. **Test substance intake:** In order to achieve the nominal daily doses of test material, the concentrations of the test substance in the diet were adjusted at each mix according to the most recent body weight data and food consumption data from the current study or from historical controls.

4. **Reproductive function:**

   a. **Estrous cycle length and periodicity:** No treatment-related effects on the stage of estrous at the time of termination were seen in either generation. The mean lengths of the

estrous cycles of females from the control, low-, mid-, and high-dose groups were respectively 4.1, 4.5, 4.2, and 4.1 days for F_0 females and 4.3, 4.2, 4.2, and 4.0 days for F_1 females. Two F_1 high-dose females did not have any complete estrous cycles during premating, and both subsequently mated but did not produce a litter. One F_0 low-dose female and one F_1 control female had abnormally long estrous cycle lengths due to prolonged diestrous, but both subsequently mated and produced litters.

b. **Sperm measures:** The mean percentages of motile and progressively motile sperm from treated F_0 and F_1 males were similar to those of their respective controls. The epididymal and testicular sperm counts of the high-dose F_0 and F_1 males were similar to those of controls. The total proportion of abnormal sperm from males in the control and high-dose groups were respectively 0.054 and 0.077 for F_0 males and 0.028 and 0.023 for F_1 males.

c. **Ovarian follicle counts:** Data from the quantitative ovarian follicle enumerations are given in Table 5. There was no evidence of treatment-related depletion of small and/or growing ovarian follicles in the high-dose F_1 females.

TABLE 5. Ovarian follicle counts from F_1 females [a]

Parameter	Nominal dose level (mg/kg bw/day)	
	Control	10
Small follicles	93±31	98±25
Growing follicles	29±9	36±9 * (124) [b]
Total	122±38	134±30

Data taken from Table 42, p. 172, MRID 45826201.

[a] Values are given as Mean ± Standard Deviation, with n = 15 for both groups.

[b] Number in parentheses equals percent of controls; calculated by reviewer.

Significantly different from controls: * $p<0.05$.

5. **Reproductive performance:** The reproductive performances of the F_0 and F_1 parental animals are summarized in Table 6. Mating, conception, fertility, and gestation indices, mean precoital intervals, and mean gestation lengths were not affected by test article administration in either generation. Mean numbers of implantation sites and grossly visible corpora lutea were not reported.

TABLE 6. Reproductive performance [a]

Observation	Nominal dose level (mg/kg bw/day)			
	Control	1	3	10
F_0 Generation (F_1 Litters)				
Number of males/females paired	30/30	30/30	30/30	30/30
Number of males/females mated ??????	0	0	0	1
Number of gravid females	25	25	25	26
Number of litters	25	25	25	26
Intercurrent deaths	0	0	0	0
Mean precoital interval (days)	2.4±1.6	2.2±1.8	2.5±2.5	2.3±0.9
Mean gestation length (days)	21.6±0.6	21.5±0.5	21.6±0.5	21.8±0.5
Male Mating Index (%)	100	100	100	96.7
Female Mating Index (%)	100	100	100	96.7
Male Conception Index (%)	83.3	83.3	83.3	89.7
Female Conception Index (%)	83.3	83.3	83.3	89.7
Male Fertility Index (%)	83.3	83.3	83.3	86.7
Female Fertility Index (%)	83.3	83.3	83.3	86.7
Gestation Index (%)	100	100	100	100
F_1 Generation (F_2 Litters)				
Number of males/females paired	29 [b]/30	30/30	30/30	30/30
Number of males/females mated	29/30	29/29	30/30	30/30
Number of gravid females	24	20	26 [c]	24
Number of litters	24	20	26	24
Intercurrent deaths	0	0	0	0
Mean precoital interval (days) [c]	2.7±1.7	2.2±1.1	2.3±1.3	2.4±1.8
Mean gestation length (days)	21.6±0.6	21.5±0.5	21.5±0.5	21.9±0.3
Male Mating Index (%)	100	96.7	100	100
Female Mating Index (%)	100	96.7	100	100
Male Conception Index (%)	82.8	69.0	86.7	80.0
Female Conception Index (%)	80.0	69.0	86.7	80.0
Male Fertility Index (%)	82.8	66.7	86.7	80.0
Female Fertility Index (%)	80.0	66.7	86.7	80.0
Gestation Index (%)	100	100	100	100

Data taken from Tables 53 and 54, pp. 183-184 and 185-186, respectively, MRID 45826201.

[a] Values are given as Mean ± Standard Deviation, as appropriate.

[b] Due to the death of one F_1 control male prior to mating, one F_1 male was paired with two females. Mating was confirmed for both females, but only one conceived and delivered.

[c] Excludes data from the F_1 mid-dose dam that was found to be gravid at necropsy.

6. Parental postmortem results:

a. Organ weights: Selected organ weight data are given in Table 7. Mean absolute and relative (to body) adrenal weights were significantly increased in F_0 and F_1 high-dose males and females and in F_1 mid-dose females. These increases were probably associated with the cytoplasmic vacuolation in the zone fasciculata, mentioned below. There were other statistically significant differences between mean absolute or relative organ weights of treated groups and controls; however, all were of small magnitude and/or were present without a dose-related trend.

TABLE 7. Adrenal weights from adult F_0 and F_1 males and pregnant females [a]

Parameter	Nominal dose level (mg/kg bw/day)			
	Control	1	3	10
F_0 Generation Males				
[Number Examined]	[25]	[25]	[25]	[25]
Terminal body weight (g)	520.1±73.3	513.9±51.4	519.0±50.2	515.7±49.1
Adrenal glands: Absolute weight (g)	0.064±0.011	0.063±0.010	0.069±0.011	0.074±0.013 * (116) [b]
Relative to body weight (g/100)	0.013±0.003	0.012±0.002	0.013±0.002	0.014±0.003 * (108)
F_0 Generation Females				
[Number Examined]	[20]	[20]	[20]	[21]
Terminal body weight (g)	289.4±25.9	283.4±17.6	291.0±20.7	287.9±15.2
Adrenal glands: Absolute weight (g)	0.081±0.010	0.075±0.012	0.082±0.010	0.092±0.015 * (114)
Relative to body weight (g/100)	0.028±0.004	0.027±0.005	0.028±0.003	0.032±0.006 * (114)
F_1 Generation Males				
[Number Examined]	[24]	[24]	[25]	[25]
Terminal body weight (g)	597.2±79.3	582.6±56.7	607.5±71.1	584.6±51.4
Adrenal glands: Absolute weight (g)	0.065±0.010	0.064±0.009	0.069±0.014	0.078±0.011 * (120)
Relative to body weight (g/100)	0.011±0.001	0.011±0.002	0.011±0.003	0.013±0.002 * (118)
F_1 Generation Females				
[Number Examined]	[19]	[15]	[21]	[19]
Terminal body weight (g)	305.5±34.0	291.4±22.4	290.1±17.8	310.1±27.4
Adrenal glands: Absolute weight (g)	0.070±0.011	0.072±0.008	0.083±0.012 * (119)	0.091±0.011 * (130)
Relative to body weight (g/100)	0.023±0.004	0.025±0.003	0.029±0.004 * (126)	0.030±0.004 * (130)

Data taken from Tables 33, 34, 35, and 36, pp. 121-122, 123-125, 126-127, and 128-130, MRID 45826201.

[a] Values given as Mean ± Standard Deviation.

[b] Numbers in parentheses equal percent of control; calculated by reviewer.

Significantly different from controls: * $p<0.05$.

b. Pathology:

1) Macroscopic examination: All reported abnormal gross observations were present at similar and/or very low incidences with no evidence of a dose-response pattern.

Gross necropsy findings from the decedent F_1 control male were limited to a dilated renal pelvis and pulmonary congestion and edema. The following gross necropsy findings were noted in the F_1 low-dose male that was sacrificed moribund: pale kidneys and a dilated renal pelvis; hemorrhage in the seminal vesicles, prostate, and adipose tissue surrounding the bladder; dark cecal contents and hemolyzed blood in the lumen of the

ileum; pale liver with a roughened surface; and dilatation of the urinary bladder with intraluminal blood and clots.

One F_1 mid-dose female had dilatation of the right uterine horn, which was filled with necrotic debris, and one necrotic fetus in the lumen of the uterus. This female had evidence of mating but never littered.

2) **Microscopic examination:** Cytoplasmic vacuolation of the zona fasciculata of the adrenal glands occurred at increased incidences and increased severity in high-dose males and mid- and high-dose females of both generations, and the incidence, but not the severity, of this finding was also increased in the F_1 mid-dose male group. Data pertaining to this lesion are given in Table 8.

One high-dose F_0 male that sired a viable litter had diffuse moderate degeneration of the seminiferous tubules of the testes in which the affected tubules had various combinations of vacuolation, degeneration, and necrosis of spermatogenic cells, and the most affected tubules were lined only by Sertoli cells. The same male also had degenerative spermatic elements comprised of numerous nucleated, multinucleated, and necrotic spermatogenic cells along with protein droplets present in both epididymides. Common findings in control and high-dose males of both generations were very slight or slight focal or multifocal degeneration of the seminiferous tubules of the testes and chronic or chronic active inflammation of the prostate.

One high-dose F_1 female that mated but failed to conceive had bilateral moderate ovarian atrophy with decreased numbers of antral follicles and corpora lutea relative to controls. This female was one of two F_1 high-dose females that did not have normal estrous cycles during premating. No lesions likely to account for reduced fertility were found in the reproductive organs from the remaining animals that failed to mate or produce a viable litter.

TABLE 8. Cytoplasmic vacuolization of the zona fasciculata of the adrenal glands in F_0 and F_1 adults

Organ/Lesion	Nominal dose level (mg/kg bw/day)							
	Males				Females			
	0	1	3	10	0	1	3	10
F_0 Adults								
Total incidence [a]	11	11	12	25 **	7	7	20 **	25 **
Average severity grade [b]	1.09	1.09	1.17	2.04	1.14	1.14	1.40	2.00
F_1 Adults								
Total incidence	14	14	21 *	25**	4	4	15 **	25 **
Average severity grade	1.36	1.57	1.43	2.36	1.00	1.00	1.27	2.00

Data taken from Tables 40 and 41, pp. 154 and 159, respectively, MRID 45826201.

[a] n = 25 for all groups.

[b] Calculated by reviewer: 1 = very slight; 2 = slight; 3 = moderate; and 4 = severe.

Significantly different from controls: * $p<0.05$; ** $p<0.01$. Fisher exact test performed by reviewer.

B. OFFSPRING:

1. **Viability and clinical signs:** Viability data for the F_1 and F_2 litters are given in Table 9. There were no treatment-related effects on postimplantation loss, mean litter size, numbers of live and dead pups, sex ratio on LD 1, or the gestation day 4 and day 21 survival indices of the F_1 and F_2 generations. There were no whole litter resorptions. One F_1 control female had an entire litter loss (of 1 pup) by day 4. Pup anogenital distances were not measured.

   No treatment-related clinical signs were reported in offspring during lactation of either generation. Litter observation data primarily included dead pups that were autolyzed or cannibalized, attached placental tissue, irregular/thin hair growth, and sporadic observations of thin, blue, cold, and/or pale pups. Structural alterations included cutis laxa in 2 F_1 control litters, filamentous tail in 1 F_1 low-dose litter, kinky tail in 1 F_1 high-dose litter, anasarca in 1 F_2 control litter, and abnormal head shape in 1 F_2 high-dose litter.

TABLE 9. Litter parameters for F_1 and F_2 litters [a]

Observation		Nominal dose level (mg/kg bw/day)			
		Control	1	3	10
F_0 dams/F_1 litters					
Number of viable litters		25	25	25	26
Number born live		311	320	335	362
Number born dead		3	5	8	1
Number [%] deaths:	Days: 0-4	32 [10.3%]	26 [8.1%]	23 [6.7%]	25 [6.9%]
	Days: 4-21	9 [4.7%]	5 [2.6%]	3 [1.5%]	6 [2.9%]
Mean live litter size:	Day 1	12.0±3.4	12.6±3.1	13.3±2.8	13.8±2.6
	Day 4 pre-cull	11.2±3.5	11.8±3.3	12.5±2.9	13.0±2.6
	Day 4 post-cull	7.5±1.1	7.6±1.0	8.0±0.0	7.8±0.7
	Day 7	7.3±1.2	7.6±1.0	7.9±0.3	7.7±0.8
	Day 14	7.2±1.3	7.6±1.0	7.9±0.3	7.7±0.8
	Day 21	7.2±1.3	7.6±1.0	7.9±0.3	7.7±0.8
Sex Ratio (% ♂)	Day 1	47	48	50	46
Postimplantation loss (mean %) [b]		12.24±17.42	6.61±8.21	7.72±11.31	7.15±7.17
Gestation survival index (%) [c]		99.0	98.5	97.7	99.7
Day 4 survival index (%) [d]		89.7	91.9	93.1	93.1
Day 21 survival index (%) [e]		95.3	97.4	98.5	97.1
F_1 dams/F_2 litters					
Number of viable litters		24	20	26	24
Number born live		312	284	356	309
Number born dead		2	3	5	3
Number [%] deaths:	Days 0-4	27 [8.7%]	29 [10.2%]	21 [5.9%]	28 [9.1%]
	Days 4-21	3 [1.6%]	2 [1.3%]	3 [1.5%]	4 [2.1%]
Mean live litter size:	Day 1	12.8±3.2	14.1±2.1	13.7±3.5	12.7±2.8
	Day 4 pre-cull	11.9±3.5	12.8±2.8	12.9±3.7	11.7±3.0
	Day 4 post-cull	7.5±1.7	7.8±0.7	7.7±1.1	7.6±0.9
	Day 7	7.5±1.7	7.8±0.7	7.6±1.1	7.6±0.9
	Day 14	7.5±1.7	7.8±0.7	7.6±1.1	7.6±0.9
	Day 21	7.5±1.7	7.8±0.7	7.6±1.1	7.6±0.9
Sex Ratio (% ♂)	Day 1	51	48	47	51
Postimplantation loss (mean %) [b]		7.04±13.04	4.88±6.50	6.99±11.24	6.34±7.05
Gestation survival index (%) [c]		99.4	99.0	98.6	99.0
Day 4 survival index (%) [d]		91.3	89.8	94.1	90.0
Day 21 survival index (%) [e]		98.4	98.7	98.5	97.9

Data taken from Tables 53, 54, 55, and 56, pp. 183-184, 185-186, 187, and 188, respectively, MRID 45826201.

[a] Values are given as Mean ± Standard Deviation, as appropriate.

[b] Postimplantation loss (%) = [(No. implants - No. viable offspring)/No. implants]×100.

[c] Gestation survival index (%) = (No. pups born alive/Total No. pups born)×100.

[d] Day 4 pup survival index (%) = (No. viable pups on day 4 pre-cull/No. born alive)×100.

[e] Day 21 pup survival index (%) = (No. viable pups on day 21/No. live pups on day 4 post-cull)×100.

2. **Body weight:** Pup body weight data for the F_1 and F_2 litters are given in Table 10. No treatment-related effects on pup body weight or body weight gain were seen in either generation.

TABLE 10. Mean pup weights from the F_1 and F_2 litters (g) [a]								
Lactation Day	Nominal dose level (mg/kg bw/day)							
	Control	1	3	10	Control	1	3	10
	Males				Females			
F_1 Pups								
1	7.1±0.7	7.1±0.7	7.2±0.6	7.2±0.6	6.8±0.6	6.7±0.8	6.8±0.6	6.8±0.7
4 pre-cull	10.5±1.4	10.4±1.7	10.5±1.1	10.3±1.1	9.9±1.3	9.8±1.4	9.9±1.0	9.8±1.2
4 post-cull	10.4±1.5	10.4±1.7	10.5±1.1	10.3±1.1	9.9±1.3	9.7±1.5	9.8±1.0	9.8±1.2
7	16.6±2.2	16.7±2.3	16.8±1.8	16.9±1.9	15.7±2.3	15.6±2.0	16.0±1.7	16.2±1.9
14	34.1±3.8	33.9±3.9	34.3±3.0	34.5±3.1	32.8±4.0	32.2±3.4	32.9±2.9	33.2±3.0
21	54.6±6.2	53.8±6.4	55.4±5.9	55.8±5.0	52.1±6.6	50.7±5.3	52.6±5.2	53.4±4.5
LD 1-21 BW Gain [b]	47.5	46.7	48.2	48.6	45.3	44.0	45.8	46.6
F_2 Pups								
1	7.0±0.9	6.6±0.8	6.9±0.7	7.4±0.8	6.5±0.7	6.2±0.7	6.6±0.8	7.0±0.6
4 pre-cull	10.2±1.2	9.7±1.2	10.0±1.5	10.7±1.6	9.6±1.1	9.1±1.1	9.7±1.6	10.3±1.6
4 post-cull	10.2±1.3	9.7±1.2	10.0±1.5	10.7±1.7	9.6±1.2	9.2±1.1	9.6±1.6	10.2±1.7
7	16.5±1.9	15.9±1.8	16.5±2.2	17.3±2.4	15.8±1.9	15.0±1.6	15.9±2.2	16.6±2.3
14	33.5±3.6	31.5±3.6	32.8±4.0	33.6±4.2	32.2±3.7	30.0±3.3	31.8±3.8	32.7±3.7
21	51.0±5.4	47.6±5.5	50.5±4.6	52.6±6.1	49.2±5.2	45.6±5.5	49.3±5.3	50.7±5.5
LD 1-21 BW Gain [b]	44.0	41.0	43.6	45.2	42.7	39.4	42.7	43.7

Data taken from Tables 57 and 58, pp. 189 and 190, respectively, MRID 45826201.

[a] Values are given as Mean ± Standard Deviation, and represent a mean of means with n = 20-26 litters.

[b] Calculated by reviewer using group mean pup weights. Not analyzed statistically.

3. **Sexual maturation** (F_1): Data pertaining to pup sexual maturation are given in Table 11. There were no treatment-related effects on preputial separation or vaginal opening.

TABLE 11. Sexual maturation of F_1 male and female pups [a]				
Observation	Nominal dose level (mg/kg bw/day)			
	Control	1	3	10
Preputial Separation				
Age (days post partum)	45.2±2.6	45.0±2.4	44.0±2.0	43.8±2.9
Body weight (g)	240.2±21.0	230.7±20.3	236.6±24.2	234.7±17.5
Vaginal Opening				
Age (days post partum)	31.2±1.9	31.2±1.3	31.5±2.1	30.8±1.4
Body weight (g)	99.8±14.0	103.3±10.5	105.8±11.5	102.8±10.8

Data taken from Tables 59 and 60, pp. 191 and 192, respectively, MRID 45826201.

[a] Values given as Mean ± Standard Deviation, with n = 30 for all groups.

4. Offspring postmortem results:

a. Organ weights: High-dose F_1 female weanlings had decreased absolute and relative (to body) spleen weights (84 and 85% of controls, respectively; $p<0.05$); however, no significant decreases were seen in the spleen weights of F_2 females or males of either generation. Other statistically significant differences were of small magnitude and/or were present without a dose-related trend.

b. Pathology:

1) **Macroscopic examination:** There were no treatment-related gross lesions. Gross necropsy findings in weanlings were limited to hydrocephalus in one high-dose F_2 male, hemorrhage in the meninges of the brain in one high-dose F_2 female, and a dark salivary gland in one high-dose F_2 female.

2) **Microscopic examination:** Organs, tissues, and lesions from the offspring were not examined histologically.

C. **ACETYLCHOLINESTERASE** The specific acetylcholinesterase activities (AChE) for erythrocytes (RBC), the brain, and the heart are given in Tables 12, 13, and 14, respectively.

1. **Erythrocyte acetylcholinesterase.** In adult rats, RBC AChE was biologically and/or statistically significantly inhibited (≥20% inhibition) in mid- and high-dose parental animals of both sexes and generations and in F_0 low-dose males. In the offspring, RBC AChE was significantly inhibited in high-dose F_1 males and females on PND 1 and 4, in high-dose F_2 males on PND 1, and in high-dose F_2 females on PND 4. Increased RBC AChE was seen in high-dose F_1 males and females on PND 22.

2. **Brain acetylcholinesterase.** High-dose adults of both sexes and generations had statistically significant decreases in brain AChE. however, none of the decreases were of sufficient magnitude to be considered biologically significant. The greatest inhibition

was seen in high-dose F_0 females (18.6% inhibition; $p<0.05$). No treatment-related effects were seen on brain AChE in offspring.

3. **Heart acetylcholinesterase.** Heart AChE was biologically or statistically significantly inhibited in high-dose F_0 males and females and in high-dose F_1 females. There were no treatment-related effects on heart AChE in offspring.

TABLE 12. Erythrocyte acetylcholinesterase (mU/mL [percent inhibition]) [a]

Generation/Age		Nominal dose level (mg/kg bw/day)							
		Control	1	3	10	Control	1	3	10
		Males				Females			
Adults									
F_0 Adults		1244±131	992±208 [20.3]	492±177 * [60.5]	240±82 * [80.7]	1200±184	992±148 * [17.3]	392±67 * [67.3]	72±52 * [94.0]
F_1 Adults		1120±202	928±117 [17.1]	344±75 * [69.3]	104±54 * [90.7]	1112±117	928±104 * [16.5]	420±110 * [62.2]	76±46 * [93.2]
Offspring									
F_1 Offspring:	PND 1 [b]	1292±139	1020±422 [21.1]	1184±230 [8.4]	820±146 * [36.5]	1044±168	1064±177	1148±248	800±183* [23.4]
	PND 4	1396±334	1436±241	1148±306 [17.8]	984±120 [29.5]	1264±458	1344±295	1300±166	992±206 [21.6]
	PND 22	1804±252	2020±180	1960±322	2320±320 *	1936±477	2048±208	1856±199	2332±230
F_2 Offspring:	PND 1 [b]	1316±226	1364±88	1472±241	976±38 * [25.8]	1148±129	1304±144	1308±220	972±84* [15.3]
	PND 4	1056±365	1332±328	1060±228	1000±173	1264±206	1424±269	1136±349	944±318 [25.3]
	PND 22	1532±227	1912±291	1604±190	1832±380	1612±303	1656±268	1512±155	1672±282

Data taken from Tables 67- 84, pp. 213-246, MRID 45826201.

[a] Values given as Mean ± Standard Deviation, with n = 5 for all groups.

[b] PND 1 samples were taken from 2 pups/sex/litter and pooled by sex and litter.

Significantly different from controls: * $p<0.05$.

TABLE 13. Brain tissue acetylcholinesterase activity (mU/mg [percent inhibition]) [a]

Generation/Age		Nominal dose level (mg/kg bw/day)							
		Control	1	3	10	Control	1	3	10
		Males				Females			
Adults									
F_0 Adults		172.6±5.0	180.0±10.9	172.7±7.3	149.6±13.2 * [13.3]	164.6±10.6	159.9±2.2	158.8±5.8	134.0±13.0 * [18.6]
F_1 Adults		154.3±9.7	150.0±5.5	146.4±6.7	135.9±8.5 * [11.9]	151.0±5.1	153.0±9.1	151.5±8.6	136.5±4.2 * [9.6]
Offspring									
F_1 Offspring:	PND 1 [b]	57.0±4.5	55.5±5.5	53.3±8.3	53.3±4.0	57.4±5.6	57.3±3.9	53.2±6.4	52.4±2.8
	PND 4	62.0±4.8	64.0±5.8	55.7±2.7	63.0±4.2	63.9±5.1	61.5±7.5	60.8±5.3	61.3±6.3
	PND 22	148.1±14.4	155.3±10.6	148.5±5.2	151.9±18.7	156.1±7.5	142.8±13.4	150.4±5.0	141.8±8.4
F_2 Offspring:	PND 1 [b]	51.9±3.7	47.6±5.0	47.9±4.8	48.5±2.7	52.0±4.2	47.8±3.2	47.1±3.7	50.8±3.9
	PND 4	61.6±5.4	62.7±9.0	64.7±3.3	65.5±5.0	62.0±2.9	59.9±5.8	67.1±5.8	66.5±4.3
	PND 22	140.0±5.8	146.9±7.2	142.7±4.9	145.3±5.4	146.6±3.1	147.0±10.8	147.8±5.1	148.0±7.9

Data taken from Tables 67- 84, pp. 213-246, MRID 45826201.

[a] Values given as Mean ± Standard Deviation, with n = 5 for all groups.

[b] PND 1 samples were taken from 2 pups/sex/litter and pooled by sex and litter.

Significantly different from controls: * $p<0.05$.

TABLE 14. Heart tissue acetylcholinesterase (mU/mg [percent inhibition]) [a]

Generation/Age		Nominal dose level (mg/kg bw/day)							
		Control	1	3	10	Control	1	3	10
		Males				Females			
Adults									
F_0 Adults		6.5±0.8	6.4±0.7	5.5±0.8 [15.4]	4.9±0.5 * [24.6]	6.4±0.9	6.6±0.7	6.3±1.2	5.0±0.5 [21.9]
F_1 Adults		5.2±0.8	5.2±0.6	4.9±0.5	4.5±0.3 [13.5]	5.8±0.5	5.4±0.5	5.0±0.6	4.5±0.6 * [22.4]
Offspring									
F_1 Offspring:	PND 1 [b]	10.7±1.4	9.4±1.1	9.8±1.0	9.4±0.9	10.0±0.9	9.2±2.3	10.1±1.2	9.4±1.3
	PND 4	7.8±1.1	7.4±0.9	7.3±0.9	7.3±0.9	7.8±0.9	8.4±0.8	8.7±1.1	8.7±0.6
	PND 22	10.2±2.0	10.0±1.9	10.2±1.4	9.8±0.9	10.0±0.9	10.0±0.8	9.8±1.4	8.7±1.5
F_2 Offspring:	PND 1 [b]	8.1±0.4	7.7±0.7	8.1±0.8	8.2±1.6	8.0±0.4	8.3±1.0	8.1±0.9	8.1±0.4
	PND 4	7.5±0.8	6.9±1.3	7.5±1.0	8.2±0.6	8.2±0.5	8.2±0.8	8.2±1.0	7.9±0.8
	PND 22	8.3±0.9	7.6±1.4	8.4±0.5	7.7±1.4	7.7±0.5	8.2±0.9	8.2±1.3	8.5±1.2

Data taken from Tables 67- 84, pp. 213-246, MRID 45826201.

[a] Values given as Mean ± Standard Deviation, with n = 5 for all groups.

[b] PND 1 samples were taken from 2 pups/sex/litter and pooled by sex and litter.

Significantly different from controls: * $p<0.05$.

II DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: According to the study author, the NOAEL for systemic parental toxicity was 1 mg/kg bw/day. This was based on increased absolute and relative adrenal weights and increases in both the incidence and the severity of cytoplasmic vacuolation of the adrenal glands at 10 mg/kg bw/day in F_0 and F_1 males and females and at 3 mg/kg bw/day in F_0 and/or F_1 females.

According to the study author, there were no treatment-related effects on reproductive performance, pup survival, or pup growth and development; therefore 10 mg/kg bw/day was considered a "NOEL" for reproductive toxicity.

The study author did not set a separate NOAEL for AChE inhibition in the red blood cells, brain, and/or heart. The study author stated that, "when brain and peripheral tissue AChE activities are known, treatment effects on RBC AChE are not considered adverse" and considered the heart tissue AChE inhibition in high-dose F_0 adult males and high-dose F_1 adult females to be a significant treatment-related effect. The study author did not consider the significantly increased RBC AChE activity in F_1 high-dose pups on PND 22 to be toxicologically significant.

B. REVIEWER (Contractor) COMMENTS:

Parental toxicity was evident as increased absolute and relative (to body) adrenal weights in high-dose males and females of both generations and in F_1 mid-dose females. These changes were correlated with an increase in the incidence and severity of cytoplasmic vacuolation of the zona fasciculata of the adrenal glands in high-dose males and mid- and high-dose females of both generations. In the F_1 mid-dose male group there was an increase in the incidence, but not the severity, of the lesion. The reviewer considers the magnitude of the increase and the apparent dose-response pattern to be consistent with a treatment-related effect despite the absence of a corresponding increase in severity. **Therefore, the LOAEL for parental systemic toxicity is 3 mg/kg bw/day, based on microscopic lesions in the adrenal glands of F_0 females and F_1 animals of both sexes. The parental systemic NOAEL is 1 mg/kg bw/day.**

There were no treatment-related effects on the gestation, day 4, or day 21 survival indices, pup body weight, clinical signs during lactation, or gross pathological observations. There were no treatment-related structural alterations. The decreased mean absolute and relative (to body) spleen weights in high-dose F_1 female weanlings may be treatment-related; however, the toxicological significance of these decreases is unknown because tissues from weanlings were not examined histologically, and hematology was not evaluated. (Note from HED reviewer: The spleen is a vascular organ and wright changes alone without histopathology support are not necessarily regarded as responses to treatment.) In the absence of treatment-related gross changes in the spleen at the necropsies of pups, weanlings, or adults of any generation and/or a similar effect on spleen weight in F_2 female weanlings or in male weanlings or adults from any generation, there is no clear evidence that this finding is

adverse. **Therefore, the LOAEL for offspring systemic toxicity is greater than the highest dose tested. The offspring NOAEL is ≥10 mg/kg bw/day.**

Mating, conception, fertility, and gestation indices, mean precoital intervals, and mean gestation lengths were not affected in either generation. There were no treatment-related effects on sperm morphology and there was no evidence of treatment-related depletion of small and/or growing ovarian follicles (which appeared to be *increased)* in high-dose F_1 females. Sexual maturation of F_1 weanlings was not affected by treatment.

Histopathological changes in reproductive organs were limited to diffuse moderate degeneration of the seminiferous tubules of the testes and moderate degenerative spermatic elements in the epididymides in one fertile F_0 high-dose male and bilateral moderate ovarian atrophy in one F_1 high-dose female that had abnormal estrous cycles, mated, but did not produce a litter. Although it is impossible to rule out an adverse effect, the incidences of these findings were quite low, and there were no indications of treatment-related decreases in fertility. No lesions likely to account for reduced fertility were found in the reproductive organs from the remaining animals that failed to mate or produce a viable litter. A second non-fertile F_1 high-dose female had an abnormal estrous cycle (prolonged diestrous) during premating, but prolonged diestrous was also seen in 1 F_1 control, and there were no treatment-related effects on mean estrous cycle length.

Therefore, the reproductive LOAEL is greater than the highest dose tested. The reproductive NOAEL is ≥10 mg/kg bw/day.

In adult rats, RBC AChE was biologically significantly inhibited (≥20% inhibition) in mid- and high-dose parental animals of both sexes and generations and in F_0 low-dose males. In the offspring, RBC AChE was inhibited in high-dose F_1 males and females on PND 1 and 4, in high-dose F_2 males on PND 1, and in high-dose F_2 females on PND 4. Increased RBC AChE was seen in high-dose F_1 males and females on PND 22.

The reviewer (Oak Ridge) disagrees with the statement by the study author that treatment-related effects on RBC AChE should not be considered adverse when brain and peripheral tissue AChE activities are known. Currently there is no standard protocol for generating data concerning AChE in the peripheral nervous system, and the heart AChE measurement done in the current study does not provide any information about AChE at other types of peripheral neuroeffector junctions, e.g. with smooth muscle, skeletal muscle, or glandular tissues.

The (contractor) reviewer also disagrees with the assessment by the study author that the significantly increased RBC AChE in high-dose F_1 pups on PND 22 was toxicologically insignificant. Such increases could be due to up-regulation of acetycholine receptors, increased synthesis of acetylcholinesterase, or some other mechanism. Cholinesterases and acetylcholine are thought to be important neuromodulators in the developing nervous system, and increased AChE and/or the resultant decrease in acetylcholine levels could have adverse effects on neurodevelopment. **Therefore, the LOAEL for erythrocyte acetylcholinesterase inhibition was 1 mg/kg bw/day, based on >20% inhibition in adult F_0 males, and the NOAEL was not identified.**

High-dose adults of both sexes and generations had statistically significant decreases in brain AChE. The greatest inhibition was seen in high-dose F_0 females (18.6% inhibition; $p<0.05$), and this may represent a threshold dose. No treatment-related effects were seen on brain AChE in offspring. **The LOAEL for brain acetylcholinesterase inhibition was not identified, and the NOAEL was 10 mg/kg bw/day.** [Note from HED reviewer: Brain AChE was shown to be reduced (10 to 19%, all $p <0.05$) for each of the four sets (F_0 and F_1 males and females), therefore, because there was consistent statistically significant inhibition, the dose level of 10 mg/kg/day is considered a LOAEL for inhibition of brain AChE.

Heart AChE was inhibited in high-dose F_0 males and females and in high-dose F_1 females. There were no treatment-related effects on heart AChE in offspring. **The LOAEL for heart acetylcholinesterase inhibition was 10 mg/kg bw/day, based on the results from adult F_0 males and females and adult F_1 females. The NOAEL is 3 mg/kg/day.**

Note from HED reviewer: Overall, the RBC, brain and heart AChE data indicate that the adult is more sensitive than the 1, 4, and 22 day old pups. Thus, no increase in sensitivity to the pups is noted in this study.

Also, since this study and DER illustrate the NOAEL and LOAELs for RBC, brain and heart AChE, decisions on which NOAEL should be used for risk assessment can be made pending resolution of policy issues and the weight of evidence from other studies with chlorpyrifos methyl.

C. STUDY DEFICIENCIES:

Plasma ChE was not assessed for. Current OPP policy does not provide that plasma ChE can be omitted from studies where ChE assessments are made. However, since this study is primarily a multi-generation reproduction study, and the lack of assessment of plasma ChE does not impact the classification with regard to assessing for reproductive performance and offspring development within the content of the reproductive study guideline.

The following deficiencies were noted but were not considered major deficiencies.

The study report did not include information regarding the stability of the test material and/or an expiration date. There was no description of the storage conditions for the test material and the diets and premixes.

The study report did not describe the ovarian sectioning and sampling procedures used in quantitative enumeration of ovarian follicle counts.

The litter of origin of each F_1 parental animal was not provided.

The summary tables of clinical signs during lactation contained an error. They stated that one F_0 control dam had a total litter loss, and there was no mention of total litter losses for F_1 dams, but according to the individual data, one F_1 control female had an entire litter loss, and there were no total litter losses for F_0 dams.

DATA FOR ENTRY INTO ISIS

Reproductive Study - rats (870.3800)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	1	3	adrenals: cytoplasmic vacuolation in z. fasciculata; incr organ wt	Parental/ systemic
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	10	not identified	none	Offspring
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	10	not identified	none	Reproductive
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	not identified	1	adults: F_0 males	RBC AChE inhibition
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	10	not identified	adults: both sexes, both gen.	Brain AChE inhibition
059102	45826201	reproductive	rats	2 generations	oral	diet	1-10	0, 1, 3, 10	3	10	adults: F_0 both sexes; F_1 females	Heart AChE inhibition

13544

R100926

Chemical:	**Chlorpyrifos-methyl**
PC Code:	**059102**
HED File Code	**13000 Tox Reviews**
Memo Date:	**06/18/2004**
File ID:	**TX0051811**
Accession Number:	**412-05-2000**

HED Records Reference Center
09/07/2004